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METHOTREXATE AS STEROID SPARING AGENT IN MYASTHENIA GRAVIS: A RETROSPECTIVE STUDY IN A SMALL COHORT OF PATIENTS

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INTRODUCTION

Myasthenia gravis (MG) is an antibody-mediated autoimmune disorder that affects neuromuscular junction (NMJ), thus resulting in fluctuating muscle weakness that is typically worsen by exertion and improved by rest.[1-3]

The incidence of myasthenia gravis ranges from 1.7 to 21 per million persons.

There is a bimodal distribution with two incidence peaks: in the third decade (strong female predominance) and in the elderly (slight male predominance). [4-6]

A recent epidemiological study in more than 2500 MG patients in Australia shows an even higher incidence of 24.9 per million person-years.[7]

Symptoms may range from ocular muscles weakness to bulbar, respiratory, and limb girdle muscles weakness. It is caused by pathogenic autoantibodies directed against the acetylcholine receptor (AChR), muscle-specific kinase (MUSK), and lipoprotein related protein 4 (LRP4) and these autoantibodies are well established as sensitive and specific diagnostic markers.[8,9]

Serum AChR positive MG is frequently associated with thymus alterations, that play a critical role in the pathogenesis of MG. The most representative thymic alteration is thymus follicular hyperplasia (TFH) and is considered the site where the autoimmune response to the AChR takes place and is maintained. On the other hand, the defect of T

cell selection seems to be responsible for the association of MG with thymoma, a tumor that can be present in 10–15% of patients.[10]

Diagnosis of MG can be confirmed by specific antibody tests and neurophysiological tests such as repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG).

Treatment of MG is based on different options:

• improvement of neuromuscular transmission by acetylcholine-esterase inhibitors, e.g. pyridostigmine

• immunosuppression (prednisone, azathioprine, methotrexate, mycofenolate)

• thymectomy

•treatment of acute exacerbations (plasmapheresis, intravenous immunoglobulin).[9]

CLINICAL PRESENTATION

Extreme fatigability is a major symptom in myasthenia gravis. The combination of fatigability with weakness of specific muscles that worsen after exercise gives strong clues to diagnosis of MG.

60% of patients present with ptosis or diplopia, or both, and in 20% of patients, the disease is restricted to ocular myastheniagravis.[11-13]

Proximal muscular weakness with bulbar muscular weakness are other prominent symptoms. Usually proximal muscle weakness is symmetrical.[14]

SUBTYPES OF MYASTHENIA GRAVIS IN RELATION TO ANTIBODIES PROFILE

1) MYASTHENIA GRAVIS WITH ACETILCHOLINE RECEPTOR ANTIBODIES

Serum acetylcholine receptor (AChR) antibodies can be present and detected by standard diagnostic testing. AChR autoantibodies are complement-activating and antigen-modulating Immunoglobulin G (IgG)1/IgG3 and can modify the NMJ causing a severe AChR loss and postsynaptic membrane simplification.

Patients with AChR antibodies may also present thymic follicular that responds to thymectomy. The age of onset of the disease shows a bimodal distribution: early onset (first symptom before 50 years old) and late onset (first symptom after 50 years old). [11,15,16]

Female cases outnumber male cases by three to one.[17,18]

This form of MG is associated with HLA-DR3, HLA-B8, and 40,41 all autoimmune disorders can be present in relatives of patients affected.[19,20]

1) MUSK-ASSOCIATED MYASTHENIA GRAVIS

MUSK is a protein expressed in the postsynaptic muscle membrane that is functionally linked to AChR and necessary to maintain AChR function. About 1–4% of patients with myasthenia gravis have serum MUSK antibodies. MUSK associated myasthenia gravis is rare in children and elder patients.[21]

This form of MG is not associated with thymus pathological changes. There is an HLA association with HLA-DQ5. [22-24]

Patient with MUSK-associated MG have a predominant involvement of cranial and bulbar muscles and suffer from dysphagia and dysarthria, they also show an important neck muscles weakness, tongue atrophy and respiratory involvement. This form of MG is a challenge for neurologist because it does not respond easily to treatment and patients need high dose of steroids to control symptoms.[25,20]

2) LRP4-ASSOCIATED MYASTHENIA GRAVIS

LRP4 is a receptor for nerve-derived agrin and an activator of MUSK and is necessary to maintain AChR function. 2-27% of patients affected by MG can show LRP4 antibodies, there is a female preponderance.[26,27]

The clinical spectrum of LRP4-MG is represented by ocular and generalized mild forms. Respiratory insufficiency occurs rarely. Thymic hyperplasia has been reported in a small number of patients affected.[28]

3) TRIPLE ANTIBODY-NEGATIVE GENERALISED MYASTHENIA GRAVIS

Patients with no detectable AChR, MUSK, or LRP4 antibodies represents an heterogenous group, some of these patients show low-affinity antibodies or low concentration of antibodies to AChR, MUSK, or LRP4 antigen targets, that are not detectable in routine assays.

4) MG ASSOCIATIED TO OTHER ANTIBODIES (very rare forms)

Recent studies noticed that other antibodies can be involved in MG (agrin, ColQ, cortactin, titin and ryanodine receptor). Their role in the disease pathogenesis is not completely known that’s why they are not currently used in diagnostic assays.

Neuronal agrin is a proteoglycan that binds LRP4 promoting the formation and activation of a tetrameric complex that binds and activates MUSK.[20,42]

At the NMJ, acetylcholinesterase (AChE) is anchored at the basal lamina by its triple collagen tail (ColQ). All ColQ-positive patients are usually women and suffer from generalized disease.[43]

Cortactin is a tyrosine kinase substrate and acts downstream of agrin/MUSK promoting AChR clustering.[44]

Antibodies directed against titin and the ryanodine receptor are strongly associated with thymoma (titin Abs are positive in 95% and ryanodine receptor Abs in 70% of thymoma patients).[45,31]

THYMOMA-ASSOCIATED MYASTHENIA GRAVIS

Thymoma-associated myasthenia gravis is considered as a paraneoplastic disease.

10-15% of patients with MG also have a thymoma and nearly all of them have detectable AChR antibodies with a generalized disease. About 30% of patients with a thymoma develop myasthenia gravis.[31]

Some data show that hyperplastic changes of myasthenia gravis thymus are related to abnormal activation of Toll-like receptors (TLRs), with production of interferons (type 1) that promote specific anti-AChR autosensitization and abnormal immune system activation.[32-34]

The occurrence of some viral infections, with a thymic viral replication (especially Epstein Barr virus and Parvovirus B19) suggests a link between TLRs signaling and B-cell activation. [35-37]

T-cell functions are also involved in MG thymus, in fact there is a reduction of regulatory T cells in MG thymomas compared with MG hyperplasia and normal thymuses and, both patients with thymoma and hyperplastic thymus show a lower number of regulatory T cells in peripheral blood.[38-40]

OCULAR MYASTHENIA GRAVIS

Some patients affected by MG have symptoms restricted to the ocular muscles and complain of diplopia with ptosis only. About the 90% of this group of patients, who complained of ocular symptoms for more than 2 years will remain in this subgroup. 50% of this group of patients can have detectable AChRantibodies.[41]

DIAGNOSIS OF MG

The diagnosis of MG should be suspected in those patients with typical symptoms such as diplopia, ptosis, dysphagia, muscle weakness and fatigability that are fluctuating and typically worsen by exertion and are improved by rest. [1-3]

All patients with a suspected MG should undergo to specific neurophysiological test such as repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG). SFEMG is considered the most sensitive test when compared to RNS which is the most specific.[9]

RNS shows the NMJ transmission defect with a decremental response of the compound muscle action potential (CMAP) to slow (2–3 Hz) motor repetitive nerve stimulation. The decremental response on slow-frequency RNS is due to failure of some muscle fibers to reach the threshold and contract.

The single fiber electromyography in patients affected by MG reveals an elongated jitter; the jitter value is the measurement of the variation of the interpotential interval between potentials of two motor fibers of the same motor unit. Patients with MG show increased jitter values. [46]

Diagnosis should be confirmed even trough specific antibody tests. The anti-AChR antibodies are highly specific for MG, in case of negativity we should search for the anti-MUSK, LRP4 or others (see above).[47]

OTHER DIAGNOSTIC TESTS

1. NEOSTIGMINE TEST

Neostigmine is a drug that blocks the active site of acetylcholinesterase, this way the enzyme cannot break down the acetylcholine molecules before they reach the postsynaptic membrane receptors.

The intramuscular injection of 0.5 mg neostigmine has a great effect on patients with MG improving clinical manifestations such as ptosis, dysarthria, limb weakness from 15 min and the effect persists for about 2 h.[9,46,47]

2. ICE PACK TEST

Cold temperature inhibits the enzyme acetylcholinesterase thus leading to a reduced breakdown of Ach at ne neuromuscular junction. This test is usually performed on patient eyelid by applying a cold ice pack on the ptotic eye for 1 to 2 minutes, an improvement of ptosis indicates a positive result.[46]

3. CT SCAN

This exam should be performed in all patient with a recent diagnosis of MG, especially patients with AChR or LRP4 autoantibodies, in order to detect the presence of a thymoma.

The negativity of the exam does not cause the diagnosis to be discarded.[47]

TREATMENT OF MYASTHENIA GRAVIS

1. SYMPTOMATIC DRUG TREATMENT

Acetylcholine esterase inhibitors inhibit the breakdown of ACh at the neuromuscular junction increasing the availability of Ach, increasing neuromuscular transmission and improving muscle weakness. Among this class of drugs pyridostigmine is the preferred one.[15]

Pyridostigmine reaches the plasma peak concentrations after 1-2 hours and its effect lasts for about 4 hours, this means that it has no long-term side effects.

Because of its cholinergic stimulation at the autonomic nervous system it can cause several side effects that may lead patients to stop treatment; side effects reported include nausea, diarrhea, cramps, increased bronchial secretions (not recommended for use in patients with asthmatic bronchopathy). There is no optimal dose, it depends on the balance between clinical improvement and adverse effects.

1. IMMUNOSUPPRESSIVE DRUG TREATMENT

a) Corticosteroids

Several studies shown that prednisone and prednisolone can improve muscle strength in patients with MG. Prednisone is activated by the liver into prednisolone. The effects usually manifest after 2–6 weeks. It is recommended to gradually increase the dose of corticosteroid in order to avoid initial deterioration.[15,48]

Recommended dose is 1.0-1.5 mg/kg per day for both prednisone and prednisolone; some authors recommend alternate-day dosing because it can reduce side-effects.[12,13]

Corticosteroids should be gradually reduced as soon as possible because of their several side effects (diabetes, hypertension, glaucoma, cataract, obesity, osteoporosis).

It is recommended to add other immunosuppressive agents in patients with MG treated with corticosteroids in order to avoid steroid related side effects.

Some studies suggest that prednisolone alone reduces the risk of developing generalized myasthenia gravis in patients with ocular MG. [49-50]

b) Azathioprine

Azathioprine (AZA) is an immunosuppressant, which inhibits DNA and RNA synthesis and interferes with T-cell function. The therapeutic response is delayed for 12-18 months, and maximal effect is obtained after 12–24 months.

It has several side effects such as flu-like symptoms or gastrointestinal disturbances (including pancreatitis that occur within the first few days of treatment). Many patients develop hepatitis with asymptomatic elevation of liver enzymes, this leads the clinician to withdrawal and often stop the therapy.

Hematological side effects are represented by leucopenia, anaemia, thrombocytopenia or pancytopenia. It is mandatory to monitor full blood cell count and liver enzymes at the beginning of therapy.[15]The recommended dose is 2–3 mg/kg per day.

AZA is widely used in combination with steroids and as steroid sparing agent, in fact some studies showed a better outcome in patients on a combination of AZA and steroids than in patients treated with steroids alone.[15,51-54]

AZA has been shown to be effective for MG in randomized placebo-controlled studies with a significant steroid-sparing activity after 15 months administration although several side effects.[51](Level A recommendation)

c) Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an inhibitor of purine nucleotide synthesis and interferes exclusively with lymphocyte proliferation. It is not recommended as first line therapy for MG even though there are several studies reporting its effectiveness alone or in combination with steroids. A retrospective study showed that MMF is effective in MG after 6 months, both with prednisone and as monotherapy.[55-56]

Other two studies (prospective and controlled trials) do not demonstrate any benefits, but these two studies had a short duration (9 months and 12 weeks).[56,57]

The recommended dose is 1.5-2 g per day and its therapeutic response is delayed as well as AZA (recommendation level B).[15] Side-effects are rare and are represented by mild headache, hemolytic anemia, nausea and diarrhea.

d) Methotrexate

Methotrexate (MTX) is an inhibitor of dihydrofolate reductase and interferes with DNA synthesis. It is widely used in many autoimmune disorders such as rheumatoid arthritis, Chron's disease and myositis.[58-60]

There are few studies on the efficacy of MTX in MG and these studies show contrasting results. Heckmann et al demonstrated that a dose of MTX of 17 mg weekly is effective on steroid-sparing in patients affected by MG and has similar effects and tolerability to AZA.[61]

Pasnoor performed a 12-month multicenter, randomized, double-blind, placebo-controlled trial of MTX 20 mg orally every week vs placebo and found no steroid-sparing benefit of MTX in MG over 12 months of treatment, despite being well-tolerated. [62]

The recommended dose is 7.5-20 mg per week. MTX has moderate side effect (stomatitis, hepatitis, leucopenia). It is recommended to supplement with folic acid.

Methotrexate should be used in selected patients with MG who do not respond to first choice immunosuppressive drugs. [15]

e) Cyclosporin

Cyclosporin inhibits calcineurin and it is used as immunosuppressive agent in autoimmune disorder as well as in patients underwent to organ transplant. [63]

A placebo-controlled double-blind randomized study in 20 patients for 6 months showed that cyclosporin improved strength compared with the placebo group.[64,65]

It has significant side effects such as nephrotoxicity and hypertension and should be considered only in patients intolerant or unresponsive to other immunosuppressant agents. [66-69]

The recommended dose is 4-6 mg/kg per day (recommendation level B).

f) Cyclophosphamide

Cyclophosphamide is an alkylating agent and acts as immunosuppressant by reducing B-lymphocyte activity and antibody synthesis, at high doses it also affects T-cells.[15]

A double-blind, placebo-controlled study on patients with MG demonstrated that Cyclophosphamide can improve muscle strength. Intravenous pulses of cyclophosphamide promote the reduction of steroids. [70]

It can be assumed orally at the dosage of 3-5 mg/Kg/ per day or through intravenous pulses (500 mg/m^{2}every 4 week).

There is a high risk of toxicity (bone marrow suppression, opportunistic infections, bladder toxicity, sterility and neoplasms), this obviously limits its use to those patients who do not response to conventional therapy (level B recommendation).[15]

g) FK506 (tacrolimus)

Tacrolimus (FK506) inhibits calcineurin and acts as Cyclosporine stopping the proliferation of activated T-cells. FK506 has also another effect, it binds ryanodine receptor thus facilitating the release of calcium from sarcoplasmic reticulum to potentiate excitation–contraction coupling in skeletal muscle.[71]

Some case reports and a small open trial all demonstrated a clinical improvement in MG with minor side effects.[72-76]

Patients affected by MG with anti-RyR antibodies showed a rapid response to FK506 treatment indicating asymptomatic effect on muscle strength in addition to the immunosuppression.[9]

The recommended dose is 3-6 mg per day and this drug should be prescribed in MG patients with poorly controlled disease or in patients with RyR antibody-positive MG (level C recommendation).[15]

h) Rituximab

Rituximab (RTX) is a chimeric IgG1 monoclonal antibody directed against CD20 antigen, it acts by reducing all type B lymphocytes. RTX has been used in different autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis. [77-79]

To the date many studies have been performed in order to assess the efficacy of RTX in MG and all confirmed its efficacy in improving symptoms, in reducing relapses in patients with refractory MG (especially anti MUSK patients) and in its steroids-sparing effects. [80-83]

RTX should be considered in patients with moderate and severe myasthenia gravis not responding to first-line treatment. Side-effects frequently reported are flu-like symptoms, nausea, vomit, hypotension, myelosuppression. A rare side effect is JC-virus-related progressive multifocal leukoencephalopathy.[20]

2. THYMECTOMY

Thymectomy is indicated in all MG patients with evidence of thymoma. A lot of studies reported a beneficial effect of thymectomy in myasthenia gravis.[11–13,15,84,85]

It is recommended to perform thymectomy early after symptoms onset. The most used methods are video-assisted thoracoscopic and robotic assisted methods.[86] Clinical improvement after thymectomy occurs gradually after some months and lasts for up to 2 years. [84]

Thymectomy is an oncological intervention and in some cases chemotherapy or radiotherapy are necessary in order to avoid local compression and spread to the thoracic cavity. [9]

Thymectomy can be performed even in patients without thymoma, its rationale stands on the pathogenic role of the thymus that has been shown in AChR-MG. [10,87,88]

A recent trial compared trans-sternal thymectomy and prednisone with prednisone alone in non-thymomatous MG. This trial proved that thymectomy was effective in both primary end-points (the time-weighted average quantitative myasthenia gravis score and the time-weighted average required dose of prednisone) and in many others secondary outcomes (azathioprine usage, hospitalization rate and proportion of patients in minimal manifestation status). [89]

3. SHORT-TERM IMMUNOMODULATION: PLASMA-EXCHANGE AND INTRAVENOUS IMMUNOGLOBULIN

The main indication for short-term immunomodulation is the treatment of MG exacerbations; other secondary indications are the treatment of deterioration after the start of steroid therapy, as preparation for thymectomy and as periodic treatment in specific cases non-responsive to conventional therapies. [54,55]

The most used methods are plasma-exchange (PLEX) (removes Abs from circulation), and intravenous immunoglobulin (IVIg) (interferes with Ab activity).

PLEX protocol consists of five exchanges sessions on alternate days and the patients need to be hospitalized for all the time; IVIg are usually administered at 2 g/kg over 2–5 days. [20]

A lot of study were performed in order to assess the efficacy of these two methods in patients affected by MG with severe exacerbations and all these studies shown that PLEX and IVIg have similar efficacy in clinical improvement, duration of effect and tolerance. [90-92]

Usually PLEX is preferred because it has a more rapid effect. There is no difference in terms of efficacy of both methods in patients with AChR-MG; patients with MUSK-MG respond well to PLEX, while the efficacy of IVIg is less consistent in this group of patients.[93]

IVIg may cause anaphylaxis in IgA-deficient patients and is contraindicated in subjects with congestive heart disease and renal insufficiency. The most reported side effects of IVIg therapy are nausea, headache and fever.

PLEX cannot be performed in patients with severe hypotension, sepsis and epilepsy. Some adverse events of PLEX are associated with vascular access (infection, thrombosis).[88]

OBJECTIVE OF THE STUDY

To the date therapy for MG is still debated and controversial: corticosteroids alone or in combination with immunosuppressive agents are the most used drugs.[47,50]

AZA has been shown to be effective for MG in randomized placebo-controlled studies with a significant steroid-sparing activity after 15 months administration although several side effects.[51]

But in clinical practice there is a consistent group of patients which cannot assume AZA because of hepatological or hematological side effects and a small group of cases, especially MUSK positive, unresponsive to AZA.

We retrospectively evaluated, in our MG cohort of over 850 patients, how many patients were treated with MTX in order to determine the efficacy, tolerability, clinical improvement and the steroid-sparing effect in our cohort.

PATIENTS AND METHODS

We found that only 15 patients (8 female and 7 male) above 850 patients, median age is about 57 years old were assuming MTX.

13 of these patients had an AChR MG and 2 of them had anti-MUSK MG and were previously treated with AZA and prednisone with uncomfortable side effects (AZA related), and because of this, patients started a combination therapy of MTX (median dose 17.5 mg per week) and PDN (median dose at onset 37 mg per day).

Two out fifteen patients assumed MTX because they also suffer from rheumatoid arthritis (RA).

Patients affected by MUSK-MG assumed a higher dose of PDN.

Demographic data, as well as pharmacological one's are shown on Table 1. (Tab.1)

Mean clinical follow up was about 8 years (from 5 to 10 years).

Each patient was evaluated 2 times per year using Quantitative Myasthenia Gravis score (QMG) and MG-Activity of daily living (MG-ADL).

QMG score is a test easy to administer and requires minimal equipment, it takes approximately 30 minutes to perform. It tests thirteen objective items on a 0 to 3 points scale (total score range 0–39), these different items are related to MG symptoms that are

double vision, ptosis, facial muscles, swallowing, speech, outstretching of both arms, forced vital capacity, hand grip (bilateral), neck muscle strength, outstretching of both legs. (Fig 1) [94]

The MG-ADL is a self-administered questionnaire composed of eight items related to symptom severity; each response to the item is graded from 0 (normal) to 3 (most severe). Two questions are about ocular symptoms, three about oropharyngeal, one about respiratory, and two about other functions (Fig. 2). Total MG-ADL scores range from 0 to 24. [95]

The scores of MG-ADL and QMG were retrospectively evaluated at the beginning of therapy (T0) and after 12 (T12), 24 (T24), 36 (T36) months and at last follow up (Tx).

Statistical analysis aimed to verify significance of the scores obtained in the five different times (T0, T12, T24, T36 and Tx) and was carried out using a non-parametric approach, since the study was pointed out on a small sample of patients.

Parameter modifications during the follow-up were performed using the Freedman and post hoc Conover test. [97]

The Mann-Whitney U test was used to compare any statistically significant differences in the QMG and MG-ADL parameters. All values of $p < 0.05$ were considered significant and the statistical, summary and inferential, post-hoc analysis were performed using the R (2) software. [98]

RESULTS

In our group of patients, the median follow-up time was about 8 years. During this period all patients underwent to MTX therapy at different dose (median dose 17.5 mg per week) and PDN (median dose at onset 37 mg per day) (see Table 1).

As shown on fig. 3 QMG score decreases significantly (P <0.001) in the five times. The post-hoc analysis allowed to highlight that this decrease is always statistically significant except for the T0-T12 period. (fig. 3)

Even MG-ADL score decreases significantly (P <0.001) in the five times (fig.4). Post-hoc analysis allowed to test that the decrease is statistically significant in all five follow-up periods.

We were able to taper successfully PDN dose starting from 37 mg to a dose of 12.5 mg per day; in eleven patients out of fifteen the dose tapering of PDN was = > 20 mg and two patients stopped PDN therapy during the period of evaluation.

DISCUSSION

MTX is widely used as an immunosuppressant in many autoimmune diseases such as Crohn's disease and psoriasis and it is first choice drug used as disease modifying agent in rheumatoid arthritis (RA). [98]

The first report about MTX and MG is from Mertens et coll, published in 1969. [99] Nevertheless recent reviews and studies on MG therapies do not include MTX as a therapeutic option or refers to MTX as a second/third choice drug in case of failure of the other immunosuppressant agents. [100-103]

As already stated, MTX is a structural analogue of folic acid, it has an anti-proliferative effect. Different studies have shown MTX safety and efficacy. A weekly MTX dose of 17-20 mg produces an optimal efficacy/toxicity ratio in RA patients. [98]

A great advantage of MTX is the weekly dosing, that is usually preferred by non-compliant patients.

There is a lack in literature about the use of MTX in patients affected with MG. There are only two trials about MTX and MG and both studies have shown controversial results. Heckmann evaluated the steroid sparing effects of 17.5 mg of MTX weekly compared with 2.5 mg/kg AZA and found that the average dose of PDN daily at month 12 was lower in the MTX-group (0.15 mg/kg prednisone) as compared with that in the AZA group in which the PDN dose daily was of 0.31 mg/kg; and the QMG scores didn't differ between two groups. [61]

Conversely Pasnoor and coll. have performed a similar trial about MTX vs placebo in AChR MG and in this group of patients the average daily PDN dose decreased in both groups with no significative difference, but MTX did not improve secondary measures (12-month changes of the Quantitative Myasthenia Gravis Score, the Myasthenia Gravis Composite Score, Manual Muscle Testing, the Myasthenia Gravis Quality of Life and the Myasthenia Gravis Activities of Daily Living of MG) compared to placebo.[62]

Our study is just a retrospective evaluation and is performed in a small cohort of patients affected by the most frequent forms of MG, AChR and MUSK MG.

We found that a long term MTX therapy is well tolerated and safe, in fact no one of the patients showed any collateral effects due to MTX therapy. No dose adjustments due to side effects were required during all the period of evaluation.

In this study 15 mg of folate was supplemented every week in order to avoid MTX side effects.[104-106]

We also found that MTX is an effective therapy in improving clinical manifestation evaluated through QMG score and MG-ADL, all 15 patients did not manifest any exacerbation of the disease during the period evaluated; and moreover, the median dose

of PDN decreased critically in our group, at the onset the PDN dose per day was 37 mg and at last follow up was 12.5 mg per day.

As shown on fig.2 MTX begins to be effective after 12 months therapy while AZA that is the most used immunosuppressant agent in MG has a delayed therapeutic response as demonstrated by two trials studying AZA as steroid-sparing immunosuppressant, in these two studies AZA become effective between 15 to 18 months. [52,107-108]

Our small retrospective analysis confirms what's already known on MTX efficacy, in fact MTX has anti-inflammatory, anti-proliferative and immune-modulatory , its immunosuppressive effects includes apoptosis and clonal deletion of activated T cells, an inhibitory effect on proliferating cells including B cells, a reduction in pro-inflammatory cytokine production by T cells and macrophages and CD95-dependant apoptosis of activated memory T cells, all these mechanism are involved in the pathogenesis of MG. [106]

The MTX recommended dose is 15 mg weekly unless the patient is more than 65 years old or his bodyweight is below 50 kg, in these specific cases the recommended dose of MTX is 7.5 mg weekly. [106]

A strong major limitation of our study is the small sample size nevertheless, our results suggest that MTX has good steroid-sparing efficacy and earlier effects if compared to AZA.

We also demonstrated MTX efficacy even in patients affected with MUSK MG, that usually has a poor outcome.

Further studies are needed in order to confirm our data, we should do future efforts in order to find others immunosuppressant agents able to improve MG disease course and

to reduce the required dose of PDN that is one of the most effective and rapid agents used in MG.

Tab. 1

	SEX	AGE	PDN DOSE T0	PDN DOSE TX	MTX DOSE
1	M	72	25 mg	17,5 mg	20 mg
2 *	F	53	60 mg	15 mg	10 mg
3 *	F	72	50 mg	30 mg	20 mg
4	M	74	25 mg	12,5 mg	20 mg
5	F	66	25 mg	5 mg	15 mg
6	M	79	50 mg	10 mg	15 mg
7	F	35	50 mg	25 mg	15 mg
8	M	58	25 mg	0	10 mg
9	F	48	75 mg	10 mg	40 mg
10	F	63	25 mg	0	7,5 mg
11	M	49	37,5mg	12,5mg	20 mg
12	F	41	25 mg	5 mg	15 mg
13	M	45	25 mg	12,5 mg	15 mg
14	F	57	37,5 mg	25 mg	15 mg
15	M	45	25 mg	12,5 mg	20 mg

Legend: * = patient with MUSK-MG; PDN = prednisone; T0 = time of beginning of the therapy; TX = time of last follow-up; MTX = methotrexate.

Fig. 1 Quantitative Myasthenia Gravis Score

QUANTITATIVE MYASTHENIA GRAVIS TESTING FORM

Patient Name: _____ Patient #: _____ Date: _____
MR#: _____ DOB: _____ Sex: ____ Ht.(in): _____ Wt.(kg): _____
Evaluator: _____ Handedness: _____ Leggedness: _____ Time of Exam: _____
Anticholinesterase Medication: _____
Comments: _____

TEST ITEMS WEAKNESS	NONE	MILD	MODERATE	SEVERE	SCORE
GRADE	0	1	2	3	
Double vision (lateral gaze) Sec.	60	11-59	1-10	Spontaneous	
Ptosis (upward gaze) Sec.	60	11-59	1-10	Spontaneous	
Facial Muscles	Normal lid closure	Complete, weak, some resistance	Complete, without resistance	Incomplete	
Swallowing 4 oz. Water (1/2 cup)	Normal	Minimal coughing or throat clearing	Severe coughing Choking or nasal regurgitation	Cannot swallow (test not attempted)	
Speech following counting aloud from 1-50 (onset of dysarthria)	None at #50	Dysarthria at #30-49	Dysarthria at #10-29	Dysarthria at #9	
Right arm outstretched (90°, sitting) Sec.	240	90-239	10-89	0-9	
Left arm outstretched (90°, sitting) Sec.	240	90-239	10-89	0-9	
Forced vital capacity	≥80%	65-79%	50-64%	<50%	
Rt hand grip: male (Kg) : female	≥45 ≥30	15-44 10-29	5-14 5-9	0-4 0-4	
Left hand grip: male (Kg) : female	≥35 ≥25	15-34 10-24	5-14 5-9	0-4 0-4	
Head, lifted (45%, supine) Sec.	120	30-119	1-29	0	
Right leg outstretched (45-50%,supine) Sec.	100	31-99	1-30	0	
Left leg outstretched (45-50%,supine) Sec.	100	31-99	1-30	0	

TOTAL MG SCORE: _____

5

Fig. 2 Myasthenia Gravis Activity of daily living (MG-ADL)

Grade	0	1	2	3	Score
Talking	Normal	Intermittent slurring or nasal speech	Constant slurring or nasal, but can be understood	Difficult to understand speech	
Chewing	Normal	Fatigue with solid food	Fatigue with soft food	Gastric tube	
Swallowing	Normal	Rare episode of choking	Frequent choking necessitating changes in diet	Gastric tube	
Breathing	Normal	Shortness of breath with exertion	Shortness of breath at rest	Ventilator dependence	
Impairment of ability to brush teeth or comb hair	None	Extra effort, but no rest periods needed	Rest periods needed	Cannot do one of these functions	
Impairment of ability to arise from a chair	None	Mild, sometimes uses arms	Moderate, always uses arms	Severe, requires assistance	
Double vision	None	Occurs, but not daily	Daily, but not constant	Constant	
Eyelid droop	None	Occurs, but not daily	Daily, but not constant	Constant	

Total score _____

Fig. 3 QMG score decrease

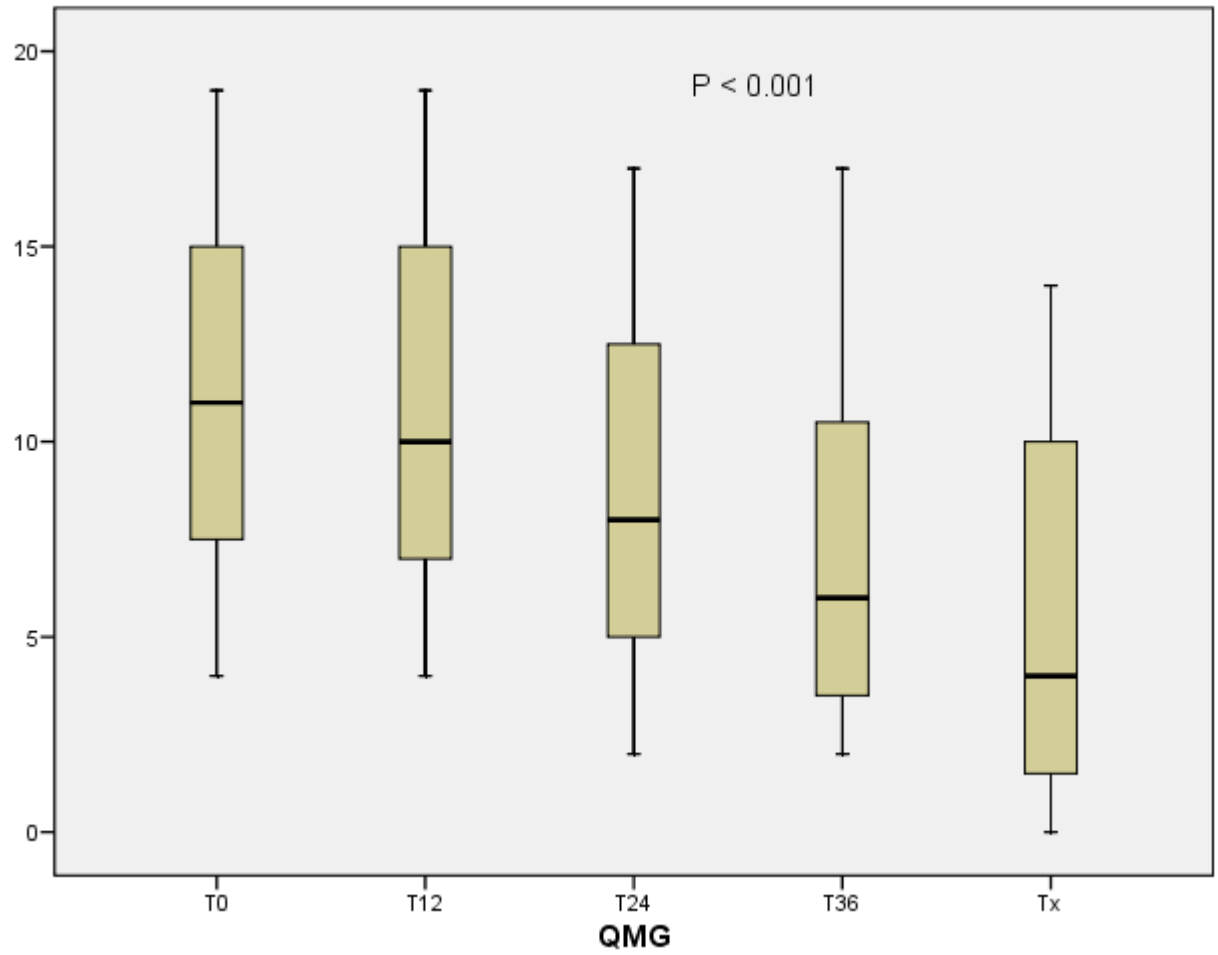
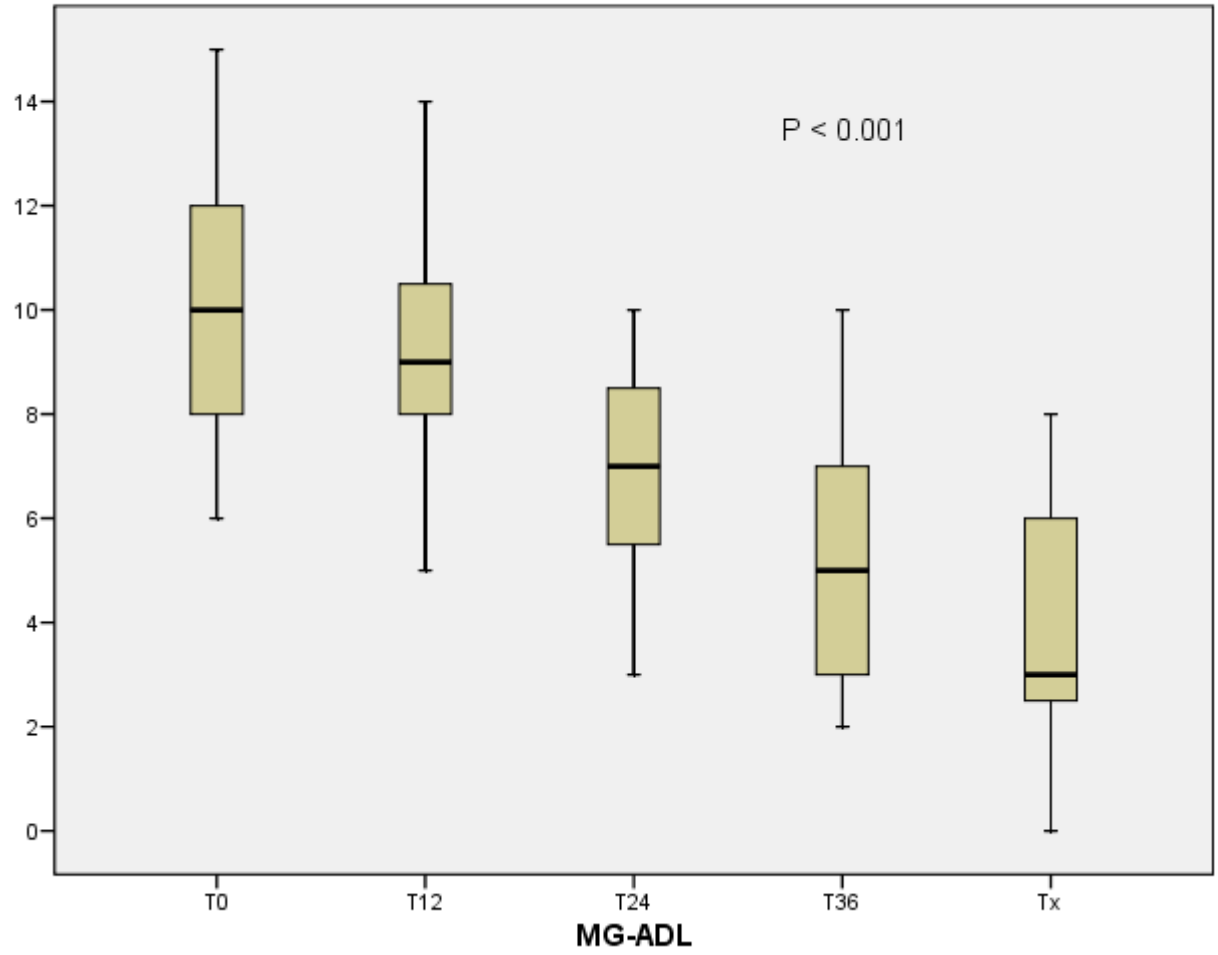


Fig. 4 MG-ADL decrease



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